The effect of maternal-induced diabetes on postnatal development of the paraventricular and ventromedial hypothalamic nuclei in albino rats: a histological, immunohistochemical, and morphometric study

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Background
The hypothalamus regulates body homeostasis by mediating endocrine, autonomic, and behavioral functions. Maternal diabetes constitutes an unfavorable environment for embryonic development.

Aim of the work
The aim of the present study was to detect the effect of maternal-induced diabetes on the structural organization of the paraventricular and ventromedial hypothalamic nuclei during postnatal development.

Methods
In this study, a total of 60 adult female albino rats were used. They were divided into two groups – group I (control group) included 30 adult female rats, and group II (experimental group) included 30 adult female rats in which diabetes was induced by a single intraperitoneal injection of alloxan monohydrate. Hypothalamic specimens of the offspring were studied at day 1, 21 days, and 2 months of age postnatally. Several techniques were used in this study, including light microscopy, which included gallocyanine–chrom–alum staining and the Golgi–Cox method, transmission electron microscopy, and immunohistochemistry. In addition, a morphometric study was carried out to estimate the number of neurons in the paraventricular and ventromedial nuclei.

Results
In the newborn, neurons in the studied hypothalamic nuclei showed presence of darkly stained nuclei. With age, these degenerative changes became more prominent. Marked reduction in the extension and branching of dendrites was observed. Electron microscopic examination of 2-month-old offspring of diabetic mothers showed the presence of chromatin condensation with many damaged mitochondria and a marked reduction in free ribosomes. Examination of the presynaptic terminals making contact with the neurons of the paraventricular and ventromedial nuclei showed marked reduction in synaptic vesicles and damaged mitochondria. In addition, an apparent increase in the number of astrocytes was found, indicating an increase in the expression of glial fibrillary acidic protein. The number of cells per area was highly decreased at 21 days and 2 months of age in the offsprings of diabetic mothers in comparison with the control group.

Conclusion
In conclusion, this study indicates that maternal diabetes affects the development and structure of the ventromedial and paraventricular nuclei. These changes suggest that there should be strict control of diabetes before and during pregnancy.

Keywords:
astrocytes, hypothalamus, maternal diabetes, paraventricular nucleus, ventromedial nucleus

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Introduction

The hypothalamus is a critical integrator of several homeostatic processes that are required for the survival of vertebrates. Disruption in the development of the hypothalamus has the potential to perturb important physiological processes with lifelong consequences [1]. Maternal diabetes constitutes an unfavorable environment for embryonic and fetoplacental development. Despite current treatments, pregnant women with either type 1 or type 2 diabetes are at increased risk of miscarriage, stillbirth, offspring congenital malformations, placental abnormalities, and intrauterine malprogramming [2]. Perinatal hyperinsulinism is pathognomonic in offspring of diabetic mothers [3].

Recently, many studies have indicated that diabetes mellitus induces brain pathological changes, named diabetic encephalopathy, which is characterized by mild

cognitive deficits and neuropathology [4]. Diabetes during pregnancy affects the health of both mothers and their infants [5].

Aim

The present study was carried out to detect the effect of maternal-induced diabetes on the structural organization of the paraventricular and ventromedial hypothalamic nuclei during postnatal development.

Materials and methods

Animal welfare committee, Asiut University approved the study. In this study, a total of 60 adult female albino rats weighing 200–300 g and aged 3 months were used. They were divided into the following two groups:

Group I (control group) included 30 adult female rats. They received no treatment.

Group II (experimental group) included 30 adult female rats in which diabetes was induced. Induction of diabetes was carried out by a single intraperitoneal injection of alloxan monohydrate (Sigma, St Louis, Missouri, USA) dissolved in 0.9% cold normal saline solution at a dose of 150 mg/kg body weight. Presence of diabetes was assessed by determining blood glucose concentrations 72 h after injection of alloxan. Rats with blood glucose levels above 200 mg/dl were then selected for the study. In both groups, mating was allowed between female and male rats. Every morning, females were examined for the presence of the vaginal plug, and vaginal smears were examined to detect the start of pregnancy. Female rats were allowed to deliver spontaneously. Their offspring were studied at day 1, 21 days, and 2 months of age.

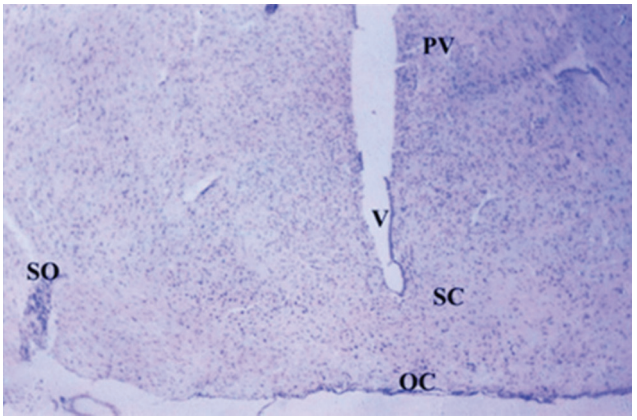
From both offspring of control mothers and offspring of diabetic mothers, six pups were studied by the gallocyanine–chrom–alum staining technique to analyze the cytoarchitecture of the paraventricular and ventromedial nuclei in the hypothalamus. Animals were anesthetized by ether inhalation. Rats were perfused in the left ventricle by normal saline followed by Bouin's fluid. Brains were extracted from the skull. Two cuts were made to determine the site of the hypothalamus. The first one cranial to the optic chiasma and the other one caudal to the mamillary bodies. The specimens were placed in Bouin's fixative solution for 12–24 h according to the age of the specimen. Three more rats were processed to study the development of cell processes by the Golgi–Cox technique. Animals were anesthetized by ether inhalation and perfused by normal saline. The brains once removed from the skulls were placed in a freshly prepared fluid containing the following:

(1) 5% potassium dichromate (16 volumes)
(2) 5% mercuric chloride (16 volumes)
(3) 5% potassium chromate (eight volumes)
(4) Distilled water (20 volumes).

The fluid was kept in a dark bottle with a cotton piece at its bottom to ensure complete immersion of the brains in the fluid. The fluid was changed after 48 h. The specimens were left in this fixative in the dark for 6 weeks. The specimens were processed according to the steps described by Drury and Wallington [6].

Five specimens from five offspring from the control and experimental groups were processed for transmission electron microscopic study. The hypothalamic specimens were dissected. They were fixed in phosphate-buffered gluteraldehyde for 24 h and postfixed in 1% osmium tetraoxide for 1 h. Semithin sections were obtained and stained with toluidine blue. Ultrathin sections (450–500 Å) from selected areas were contrasted with uranyle acetate and lead citrate and photographed at Assiut University Electron Microscopic Unit. Immunohistochemical study using anti-glial fibrillary acidic protein (GFAP) was performed at 2 months of age to demonstrate astrocytes. Morphometric study was carried out on gallocyanine-stained sections to estimate the number of neurons in the paraventricular and ventromedial nuclei both in the offspring of control mothers and in the offspring of diabetic mothers in all the studied groups using an image analysis system (Leica Q500 MC, Leica Microsystems Germany) that proposed counting cells per specific area (12 360 μm^2). Statistical analysis using *t*-test was performed to compare experimental groups with control groups. The research was reviewed and approved by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University and the approval date was 16 of July 2011.

Figure 1



A photomicrograph of a coronal section in the anterior region of the hypothalamus of two months old rat offspring of control mother showing paraventricular nucleus (PV), suprachiasmatic nucleus (SC), supraoptic nucleus (SC). Third ventricle (V) and optic chiasma (OC). (Gallocyanin stain ×40).

Results

General structure of the hypothalamus

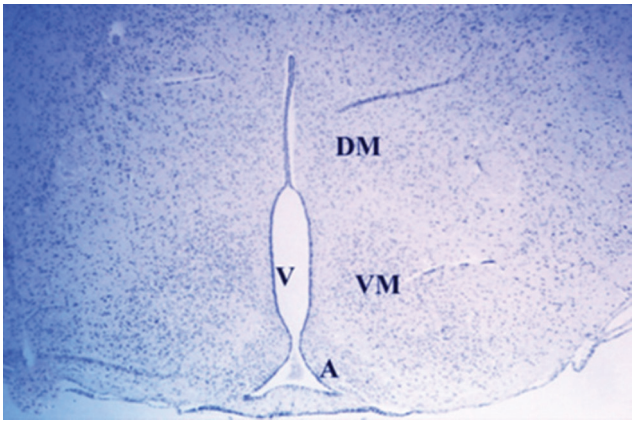
The anterior region of the hypothalamus shows the presence of the paraventricular nucleus which is situated bilaterally (Fig. 1). At the tuberal region of the hypothalamus the ventromedial nucleus can be distinguished (Fig. 2).

The paraventricular nucleus

Control group

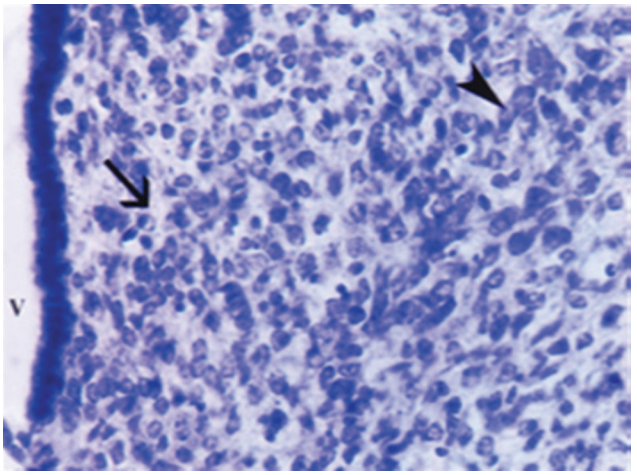
In newborn rats, light microscopic examination revealed that the paraventricular nucleus is distinctly differentiated from the surrounding tissue. It starts to differentiate into a dorsolateral (magnocellular) part consisting of cells of variable shape and a ventromedial (parvocellular) part consisting of smaller, rounded cells separated from the lining of the third ventricle by a space (Fig. 3). Electron microscopic examination of the neurons in the magnocellular part of the nucleus revealed that the cells have rounded euchromatic nucleus with well-defined nucleolus. The cells had abundant cytoplasm, which contained numerous free ribosomes, rough endoplasmic reticulum cisternae, mitochondria, and some vesicles (Fig. 4). Examination of the synaptic contacts with these neurons showed that the presynaptic terminals have numerous synaptic vesicles and mitochondria with well-defined cresta (Fig. 4, inset).

Figure 2



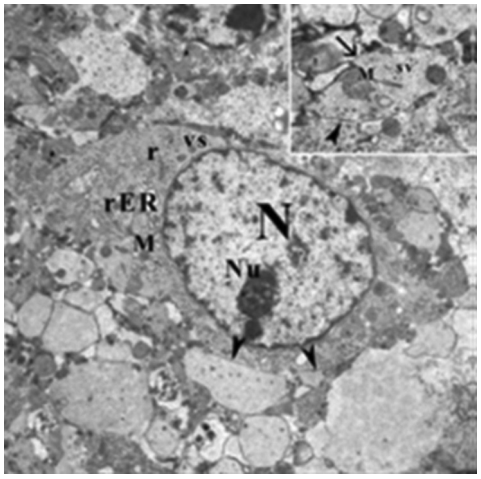
A photomicrograph of a coronal section in the tuberal region of the hypothalamus of two months old rat offspring of control mother showing the arcuate nucleus (A), ventromedial nucleus (VM) and dorsomedial nucleus (DM). Third ventricle (V). (Gallocyanin stain ×40).

Figure 3



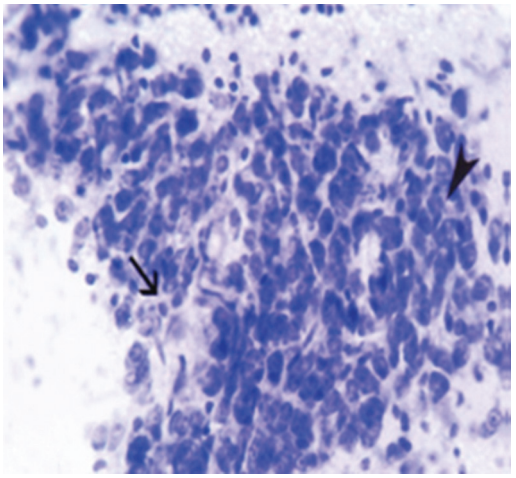
A photomicrograph showing the paraventricular nucleus in the hypothalamus of newborn rat offspring of control mother. The nucleus is divided into two parts, dorsolateral (magnocellular) part (arrow head) and ventromedial (parvocellular) part (arrow). (Gallocyanin stain x 400).

Figure 4



An electron photomicrograph showing the cells of the magnocellular part in the paraventricular nucleus in hypothalamus of newborn rat offspring of control mother, the neurons have rounded euchromatic nucleus (N) with prominent nucleolus (Nu). The cytoplasm contains numerous free ribosomes (r), rough endoplasmic reticulum (rER), mitochondria (M) and some vesicles (vs). Note the presence of many synaptic contacts with the cell (arrow heads). (x4800). Inset: Shows synaptic contact (arrow head) between the presynaptic nerve terminal (arrow).

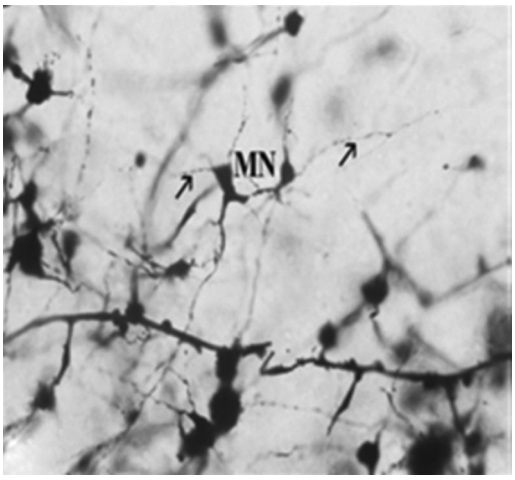
Figure 5



A photomicrograph showing the paraventricular nucleus in the hypothalamus of twenty-one days old rat offspring of control mother. The nucleus is divided into two parts magnocellular part (arrow head) and parvocellular part (arrow). (Gallocyanin stain ×400).

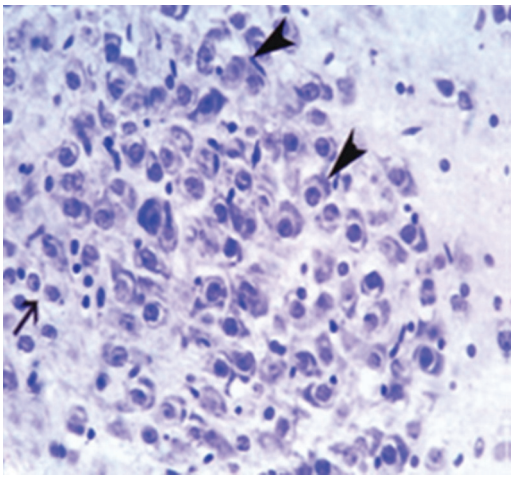
In 21-day-old rats, the nuclei were well differentiated into the magnocellular part and the parvocellular part. The magnocellular part consisted of large-sized cells of variable shape. The parvocellular part consisted of scattered, small-sized cells (Fig. 5). The nucleus had many multipolar neurons. The neurons had long extended dendrites with numerous spines (Fig. 6). Ultrastructural examination showed that the cells in the magnocellular part had oval nucleus with fine, dispersed chromatin and indented nuclear membrane. The cytoplasm showed the presence of numerous free ribosomes, rough endoplasmic reticulum cisternae, and mitochondria (Fig. 7). Many synaptic contacts with neurons can be observed (Fig. 7, inset).

Figure 6



A photomicrograph of the paraventricular nucleus in the hypothalamus of twenty-one days old rat offspring of control mother. Note multipolar neuron (MN) with extension and branching of dendrites that carry many spines (arrows) (Golgi-Cox stain ×250).

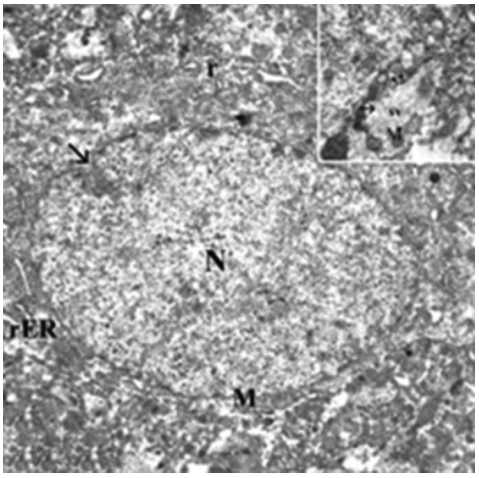
Figure 8



A photomicrograph showing the paraventricular nucleus in the hypothalamus of two months old rat offspring of control mother. The nucleus is divided into two parts magnocellular part consists of large polyhedral cells filled with Nissl granules (arrow heads) and parvocellular part consisting of small rounded cells (arrow). (Gallocyanin stain ×400).

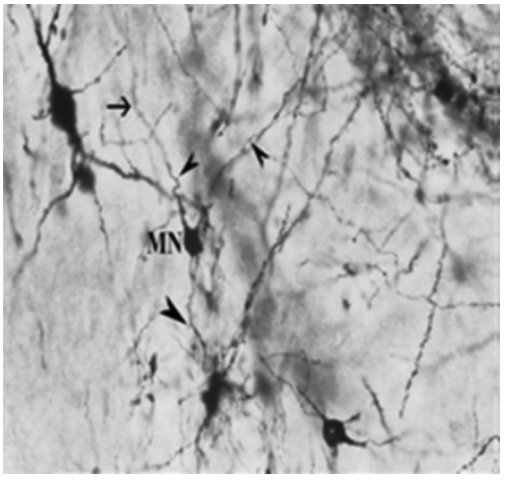
At the age of 2 months, the magnocellular part of the paraventricular nucleus consisted of large cells; they appeared to be widely separated compared with the 21-day-old rats. They were of variable shape and size. They appeared to be filled with Nissl granules. Their nuclei were rounded with well-defined nucleoli. The parvocellular part had small rounded cells, which were widely separated (Fig. 8). Many multipolar neurones were be observed in the paraventricular nucleus. The cells had long extended branching dendrites, which carried many spines (Fig. 9). Immunohistochemical

Figure 7



An electron photomicrograph showing the cells of the magnocellular part in the paraventricular nucleus in hypothalamus of twenty-one days old rat offspring of control mother. The cells have oval nucleus with fine dispersed chromatin (N). Arrow points to indentation of nuclear membrane. The cytoplasm contains a lot of free ribosomes (r), rough endoplasmic reticulum cisternae (rER) and mitochondria (M). (x5800). Inset: Shows synaptic contact (arrow head) on the surface of cells of the paraventricular nucleus.The presynaptic terminal (arrow) shows the presence of a lot of synaptic vesicles (sv) and mitochondria (M). (x19000).

Figure 9



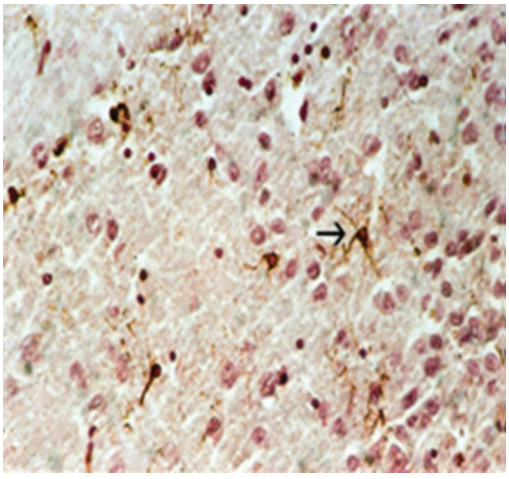
A photomicrograph of the paraventricular nucleus in the hypothalamus of two months old rat offspring of control mother. It shows many multipolar neurons (MN) with long extended dendrites (arrows) which carry numerous spines (arrow heads). (Golgi-Cox stain ×250).

staining using anti-GFAP demonstrated the presence of a few astrocytes in the paraventricular nucleus, which appeared to be star-shaped with branching processes between neurons (Fig. 10). Ultrastructural examination showed that the cells in the magnocellular part had large nucleus with fine granular chromatin. The cytoplasm showed the presence of free ribosomes, rough endoplasmic reticulum, and mitochondria (Fig. 11). At the site of synaptic contact, the presynaptic terminal showed the presence of many synaptic vesicles and mitochondria with well-defined cristae (Fig. 11, inset).

Experimental group

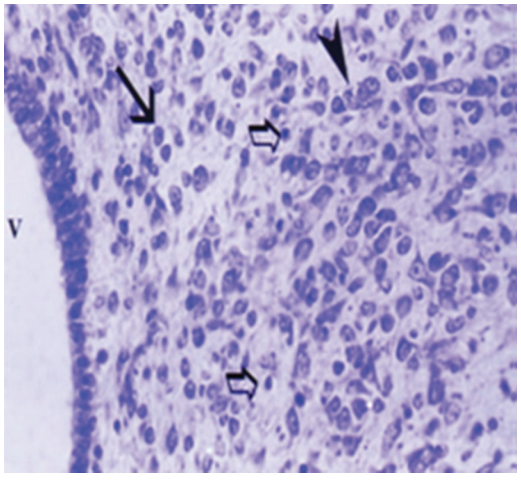
In the newborn, light microscopic examination showed that both the magnocellular part and the parvocellular part of the paraventricular nucleus showed the presence of degenerated neurons. The cells appeared to be lightly stained, which indicates loss of Nissl granules. Some cells with darkly stained nuclei were also observed (Fig. 12). Electron microscopic examination of the magnocellular neurons revealed peripheral chromatin condensation. The cytoplasm contained dilated rough endoplasmic reticulum cisternae, some vacuoles, and dense bodies (Fig. 13). Examination of

Figure 10



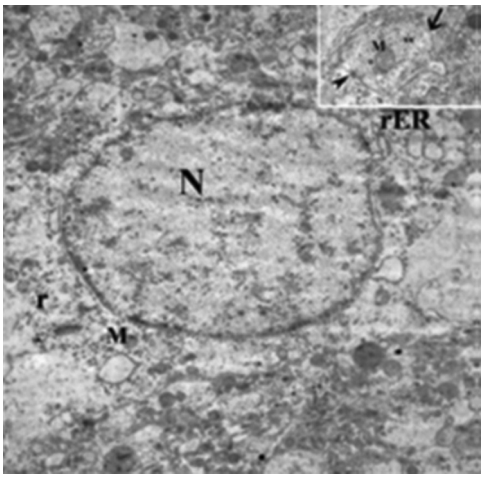
A photomicrograph of the paraventricular nucleus in the hypothalamus of two months old rat offspring of control mother. It shows the presence of few scattered astrocytes (arrows) (Anti GFAP immunostaining ×400).

Figure 12



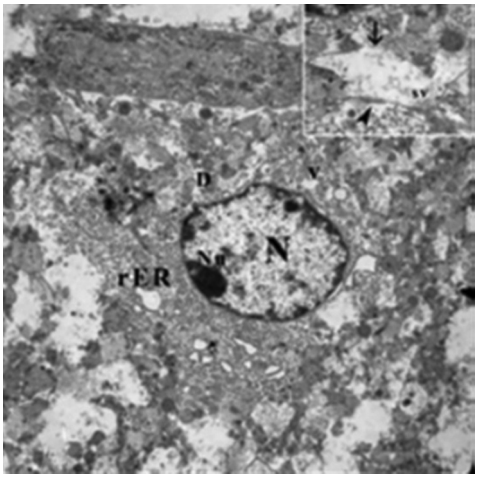
A photomicrograph showing the paraventricular nucleus in the hypothalamus of newborn offspring of diabetic mothers. The magnocellular part (arrow head) and parvocellular part (thin arrow) show that cells are widely separated and lightly stained. Some cells have darkly stained nuclei (big arrow). (Gallocyanin stain x 400).

Figure 11



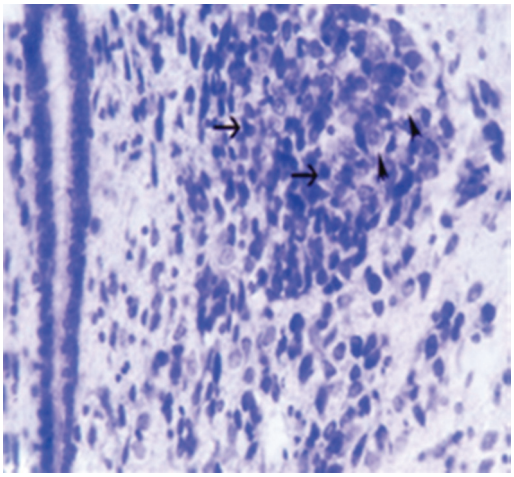
An electron photomicrograph of a cell in the magnocellular part in the paraventricular nucleus in hypothalamus of two months old rat offspring of control mother shows rounded nucleus (N) with fine granular chromatin. The cytoplasm shows the presence of free ribosomes (r), rough endoplasmic reticulum cisternae (rER) and mitochondria (M). (x5800). Inset: Shows synaptic contact (arrow head) on the cells of paraventricular nucleus. Note the presence of numerous synaptic vesicles (sv) and mitochondria (M) with well-defined cristae in the presynaptic terminal (arrow). (x19000).

Figure 13



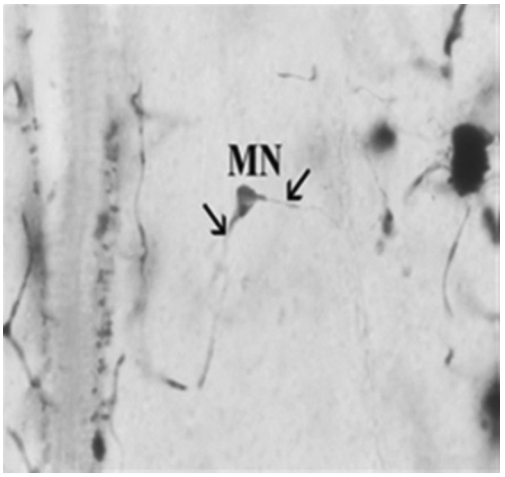
An electron photomicrograph showing the cells of the magnocellular part in the paraventricular nucleus in hypothalamus of newborn offspring of diabetic mother, the nucleus has peripheral chromatin condensation (N). Dilated rER cisternae, some vacuoles (v) and dense bodies (D) can be observed in the cytoplasm. (x4800).Inset: Shows synaptic contact (arrow head) between the presynaptic nerve terminal (arrow) and the magnocellular neurons in the paraventricular nucleus. Marked decrease in the amount of synaptic vesicles (sv) can be observed. (x19000).

Figure 14



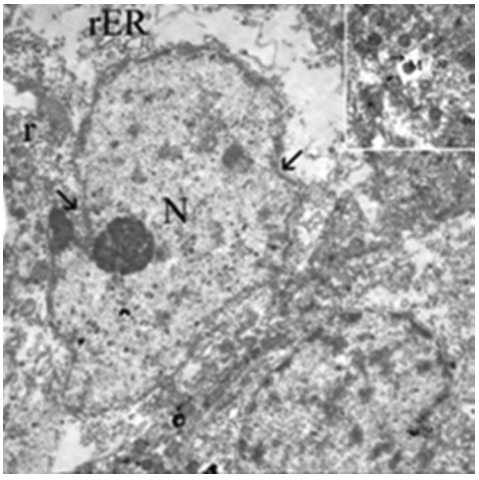
A photomicrograph showing the paraventricular nucleus in the hypothalamus of twenty- one days old offspring of diabetic mothers. Note the presence of many neurons with darkly stained nuclei (arrows), some cells with faintly stained nuclei (arrow heads) can be seen.(Gallocyanin stain ×400).

Figure 15



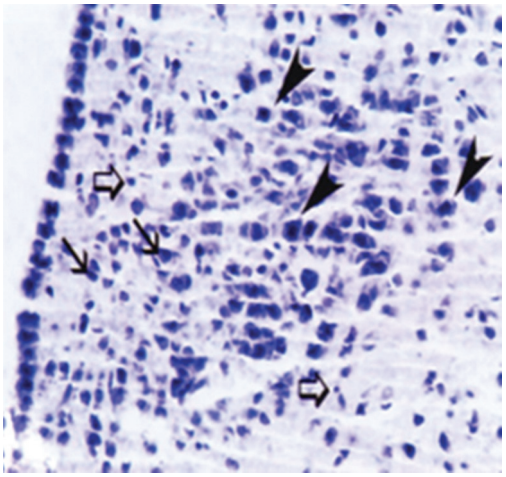
A photomicrograph of the paraventricular nucleus in the hypothalamus of twenty-one days old rat offspring of diabetic mother shows the presence of multipolar neuron (MN). Note the decrease in the extension and branching of dendrites (arrows). (Golgi-Cox stain x250).

Figure 16



An electron photomicrograph of the cells of the magnocellular part in the paraventricular nucleus in hypothalamus of twenty-one days old offspring of diabetic mother. The nucleus (N) shows marked irregularities of the nuclear membrane (arrows) and patchy areas of chromatin condensation. Marked loss of free ribosomes and dilated rER cisternae can be observed. (x5800). Inset: Shows synaptic contact (arrow head) on the surface of cells of the paraventricular nucleus. Note the marked loss of synaptic vesicles in the presynaptic terminal (arrow). Mitochondria (M). (x19000).

Figure 17



A photomicrograph showing the paraventricular nucleus in the hypothalamus of two months old offspring of diabetic mothers. Note marked decrease in size of the nucleus than in the control rat. Both magnocellular (arrow heads) and parvocellular (thin arrow) parts show cells with darkly stained nuclei. Many cells have pyknotic nuclei (big arrows). (Gallocyanin stain x 400).

the presynaptic terminals making contact with these neurons showed an apparent decrease in the amount of synaptic vesicles (Fig. 13, inset).

At 21 days of age, the magnocellular and parvocellular parts of the paraventricular nucleus showed the presence of many cells with darkly stained nuclei and lysis of the cytoplasm. In addition, some cells with faintly stained nuclei were found to be present (Fig. 14). The neurons showed decreased extension and branching of dendrites (Fig. 15). Ultrastructural examination showed that in the magnocellular part the nucleus has marked irregularities of the nuclear membrane with patchy areas of chromatin condensation. The cytoplasm had marked loss of free ribosomes and dilated rough endoplasmic reticulum (rER) cisternae (Fig. 16). At the site of synaptic contact, the presynaptic terminal making contact with the cells showed a marked decrease in the amount of synaptic vesicles (Fig. 16, inset).

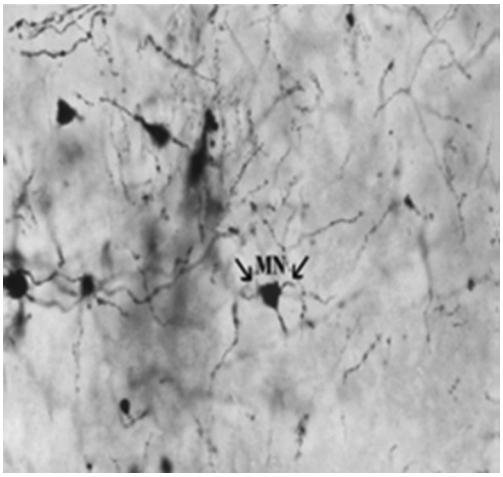
At the age of 2 months, most of the cells had pyknotic nuclei in both magnocellular and parvocellular parts of the nucleus (Fig. 17). The neurons showed a decrease in extension and branching of the dendrites (Fig. 18). An apparent increase in the

Table 1 The mean number of cells in the paraventricular nucleus per an area of 12 360 μm² of the offspring of control mothers and that of diabetic mothers at different ages

Ages	Offspring of control mothers			Offspring of diabetic mothers			Difference	*t*
	n	Mean	SE	*n*	Mean	SE		
Newborn	5	106.20	2.083	5	101	1.183	5.200	2.170
21 days	5	165.60	2.040	5	126.80	1.985	38.800	13.633**
2 months	5	125	1.844	5	89.80	1.068	35.200	16.520**

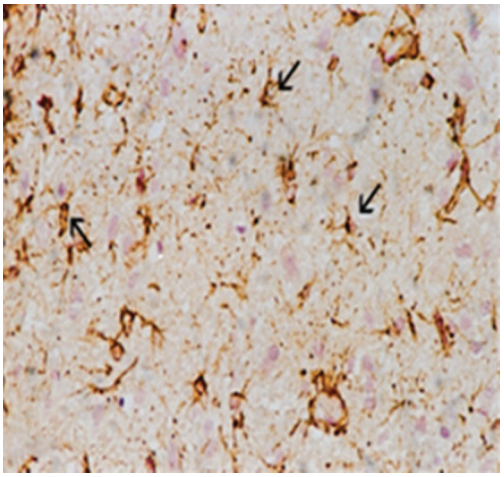
**$P<0.01$, highly statistical significance difference.

Figure 18



A photomicrograph of the paraventricular nucleus in the hypothalamus of two months old rat offspring of diabetic mother. Note the marked decrease in the extension and branching of dendrites (arrows) in a multipolar neuron(MN). (Golgi- Cox stain x250).

Figure 19



A photomicrograph showing the paraventricular nucleus in the hypothalamus of two months old rat offspring of diabetic mother. Note the apparent increase in astrocytes in comparison with the control (arrows). (Anti GFAP immunostaining ×400).

number astrocytes in comparison with the control can be observed, indicating an increase in the expression of GFAP within the paraventricular nucleus (Fig. 19). Ultrastructurally, cells in the magnocellular part showed swelling of the nuclear membrane and peripheral chromatin condensation. The cytoplasm showed an apparent decrease in the amount of free ribosomes and damaged mitochondria (Fig. 20). At the site of synaptic contact, the presynaptic terminal showed a decrease in synaptic vesicles and condensed mitochondria (Fig. 20, inset).

Morphometric analysis

In newborn rats, the mean number of cells in the paraventricular nucleus per an area of 12 360 μm² in the treated group was 101 ± 1.183, which showed an insignificant difference ($P > 0.05$) when compared with the control group in which the mean number was 106.2 ± 2.083. At the ages of 21 days and 2 months, the mean numbers of cells in the offspring of diabetic mothers were 126.8 ± 1.985 and 89.80 ± 1.068, respectively, a highly significant decrease ($P < 0.01$) when compared with the control groups in which the mean numbers were 165.60 ± 2.04 and 125 ± 1.844, respectively (Table 1 and Histogram 1).

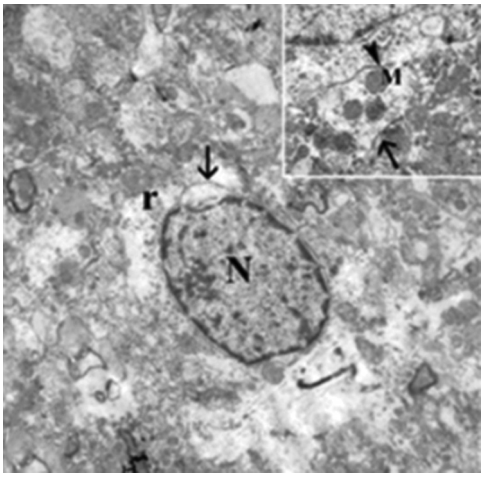
The ventromedial nucleus

Control group

In newborn rats, light microscopic examination revealed that the ventromedial nucleus starts to get its typical egg-shaped appearance (Fig. 21). The cells of the ventromedial nucleus are of variable size and shape with prominent nucleoli (Fig. 21). Electron microscopic examination showed that the cells have oval euchromatic nuclei with fine, dispersed chromatin. The cytoplasm is rich in free ribosomes, rER cisternae, and mitochondria (Fig. 22). The presynaptic terminal at the site of synaptic contact showed the presence of numerous synaptic vesicles and mitochondria (Fig. 22, inset).

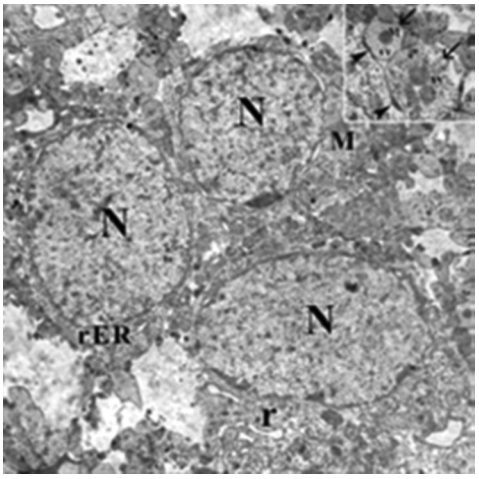
At the age of 21 days, gallocyanine-stained sections showed that it consists of variably shaped and sized cells with vesicular nuclei and prominent nucleoli (Fig. 23). The Golgi–Cox examination showed the presence of multipolar neurons with extended branched dendrites with many spines (Fig. 24). Ultrastructural examination showed that the cells had rounded nucleus with evenly distributed chromatin. The cytoplasm contained many rER cisternae, free ribosomes, and mitochondria (Fig. 25). We also observed the presence

Figure 20



An electron photomicrograph of a cell in the magnocellular part in the paraventricular nucleus in hypothalamus of two months old offspring of diabetic mothers shows that the nucleus has peripherally condensed chromatin (N), swollen nuclear membrane (arrow). Note the decrease in free ribosomes (r). (x5800) Inset: Shows synaptic contact (arrow head) on the cells of paraventricular nucleus. Note the loss of synaptic vesicles in the presynaptic terminal (arrow) and condensed mitochondria (M). (x19000).

Figure 22

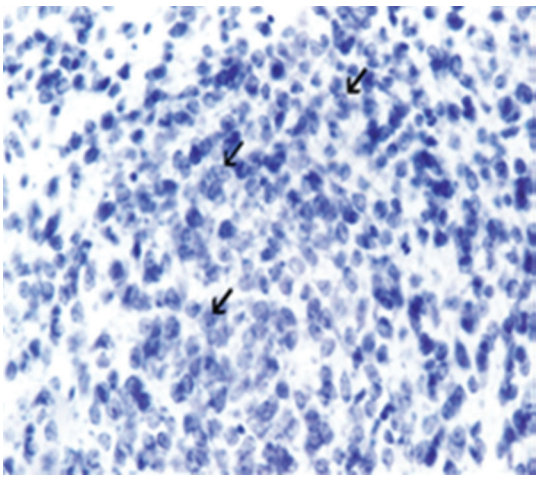


An electron photomicrograph showing the cells of the ventromedial nucleus in hypothalamus of newborn rat offspring of control mother. The cells have oval nuclei with fine dispersed chromatin (N).The cytoplasm is rich with free ribosomes (r), rough endoplasmic reticulum cisternae (rER) and mitochondria (M). (x4800) Inset: Shows the presence of many synaptic contacts (arrow heads). (x19000).

of many synaptic contacts with the neurons of the ventromedial nucleus. Many synaptic vesicles and mitochondria were present in the presynaptic terminals making contact with these neurons (Fig. 25, inset).

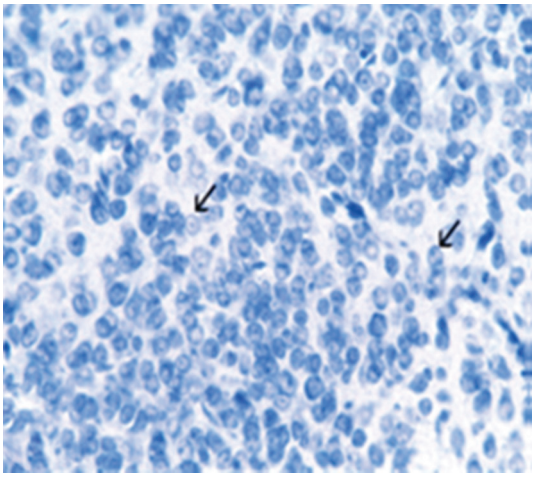
At the age of 2 months, the cells of the ventromedial nucleus appeared to be variable in shape and size. They had vesicular nuclei and prominent nucleoli (Fig. 26). The nucleus had many multipolar neurons, with long, extended, and branching dendrites. These dendrites carried many spines (Fig. 27). Immunohistochemical

Figure 21



A photomicrograph showing the ventromedial nucleus in the hypothalamus of newborn rat offspring of control mother. The cells have well defined nuclei with prominent nucleoli (arrows). (Gallocyanin stain ×400).

Figure 23



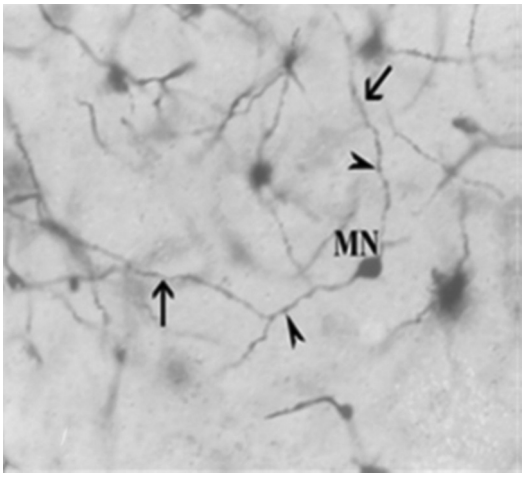
A photomicrograph showing the ventromedial nucleus in the hypothalamus of twenty-one days old rat offspring of control mother. The nucleus consists of cells with variable shape and size with vesicular nuclei (arrows). (Gallocyanin stain ×400).

staining using anti-GFAP demonstrated the presence of a few scattered astrocytes (Fig. 28). Ultrastructural examination showed that the cell had rounded nucleus with fine, dispersed chromatin and prominent nucleolus. The cytoplasm was rich in free ribosomes, rough endoplasmic reticulum cisternae, and mitochondria. Many Golgi complexes were also present (Fig. 29). The neurons showed the presence of many synapses, which had numerous synaptic vesicles and mitochondria in the presynaptic terminals making contact with it (Fig. 29, inset).

Experimental group

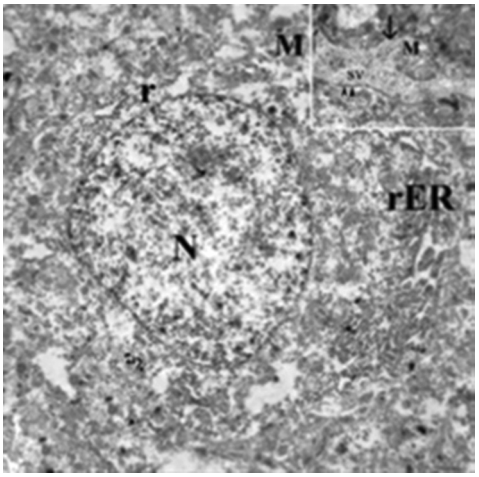
In newborn rats, the ventromedial nucleus showed the presence of some cells with darkly stained

Figure 24



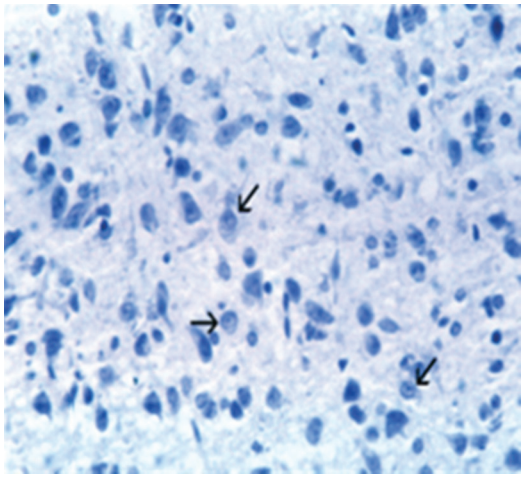
A photomicrograph showing the ventromedial nucleus in the hypothalamus of twenty- one days old rat offspring of control mother. It shows the presence of multipolar neuron (MN) with extended and branched dendrites (arrows). Note the presence of spines on the dendrites (arrow heads). (Golgi-Cox stain ×250).

Figure 25



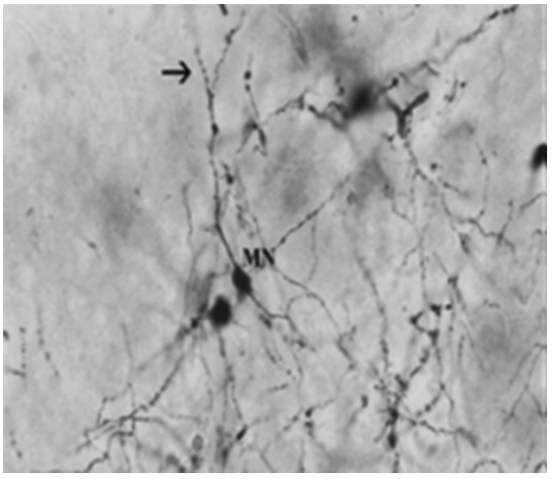
An electron photomicrograph of the ventromedial nucleus cell in hypothalamus of twenty-one days old rat offspring of control mother. The cell has rounded nucleus with evenly distributed chromatin (N). The cytoplasm contains a lot of rough endoplasmic reticulum cisternae (rER), free ribosomes (r) and mitochondria (M). (x4800) Inset: Shows a synaptic contact (arrow head) on the cells of the ventromedial nucleus in hypothalamus of control twenty one days old rat. Note the presence of numerous round synaptic vesicles (sv) and mitochondria (M) in presynaptic terminal (arrow) (x19000).

Figure 26



A photomicrograph showing the ventromedial nucleus in the hypothalamus of two months old rat offspring of control mother. The nucleus consists of cells with variable shape.They have rounded nuclei with prominent nucleoli (arrows) (Gallocyanin stain ×400).

Figure 27



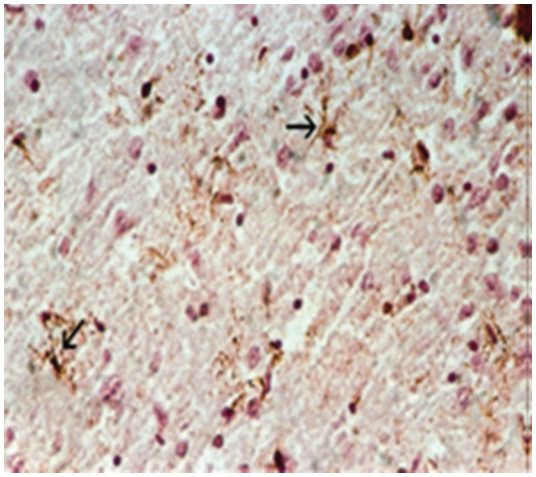
A photomicrograph showing the ventromedial nucleus in the hypothalamus of two months old rat offspring of control mother. Note that the cells (MN) have long extended and branched dendrites with numerous spines (arrow). (Golgi-Cox stain ×250).

nuclei (Fig. 30). Electron microscopic examination of the cells revealed that the cytoplasm contained many vacuoles, dense bodies, and dilated rough endoplasmic reticulum. The free ribosomes were not seen (Fig. 31). At the site of synaptic contact, the presynaptic terminal showed the loss of synaptic vesicles and the presence of damaged mitochondria (Fig. 31, inset).

At the age of 21 days, the nucleus showed the presence of many cells with cytoplasmic lysis and darkly stained nuclei. In addition, the cells appeared to be less in number in comparison with the control rats (Fig. 32). In addition, the neurons showed a marked decrease in extension and branching of dendrites (Fig. 33). Ultrastructurally, the cell showed marked irregularities of the nuclear membrane, the cytoplasm appeared rarefied, and there was a decrease in the number of free ribosomes besides many dense bodies (Fig. 34). Apparent loss of synaptic vesicles can be observed in the presynaptic terminals making contact with the neurons (Fig. 34, inset).

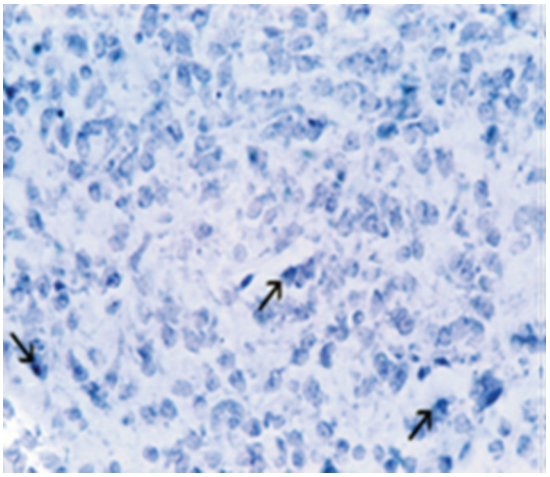
At the age of 2 months, many cells in the ventromedial nucleus appeared to be faintly stained, with some cells showing the presence of pyknotic

Figure 28



A photomicrograph showing the ventromedial nucleus in the hypothalamus of two months old rat offspring of control mother. Few astrocytes can be observed (arrows). (Anti GFAP immunostaining ×400).

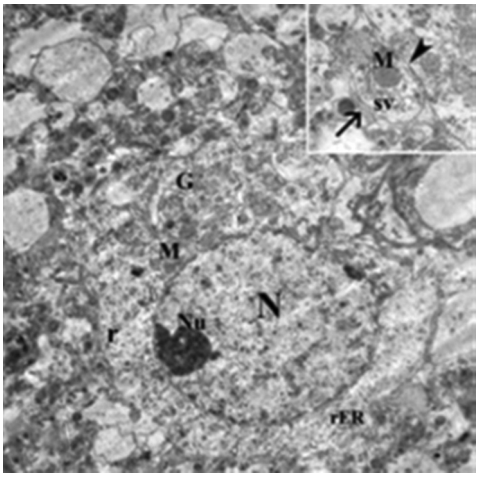
Figure 30



A photomicrograph showing the ventromedial nucleus in the hypothalamus of newborn offspring of diabetic mothers. The cells are dispersed and lightly stained. Note the presence of nerve cells with darkly stained nuclei (arrows). (Gallocyanin stain ×400).

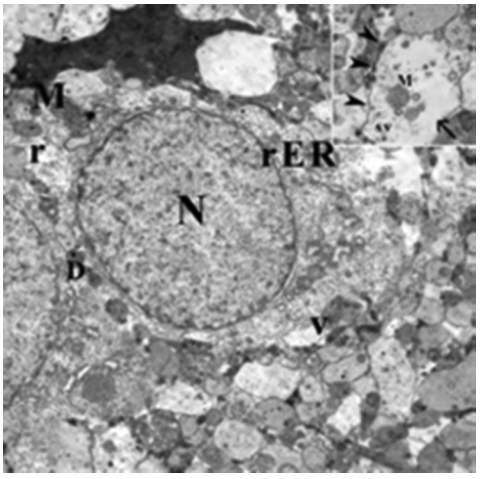
nuclei and complete lysis of the cytoplasm (Fig. 35). The Golgi–Cox examination showed a decrease in extension and branching of dendrites (Fig. 36). Immunohistochemical staining using anti-GFAP demonstrated the presence of many astrocytes, which had branched processes in comparison with the control. This indicates the increase in the expression of GFAP (Fig. 37). Electron microscopic examination of the cells showed peripheral chromatin condensation of the nuclei. The cytoplasm appeared rarified and had a few free ribosomes, multivesicular bodies, and dilated rough endoplasmic reticulum cisternae (Fig. 38). Examination of the presynaptic terminal showed loss of the synaptic vesicles, and the mitochondria showed destruction of their cristae (Fig. 38, inset).

Figure 29



An electron photomicrograph of the ventromedial nucleus cell in hypothalamus of two months old rat offspring of control mother shows a rounded nucleus with evenly distributed chromatin (N) and well defined nucleolus(Nu).The cytoplasm is rich in free ribosomes (r), rough endoplasmic reticulum cisternae (rER), Golgi apparatus (G) and mitochondria (M).(x4800) Inset: Shows synaptic contact (arrow head) on the cells of ventromedial nucleus in hypothalamus of control two months old rat. Note the presence of numerous synaptic vesicles (sv) and mitochondria (M) in presynaptic terminal (arrow). (x19000).

Figure 31



An electron photomicrograph showing the cells of the ventromedial nucleus in hypothalamus of newborn rat offspring of diabetic mother. The cytoplasm contains vacuoles (v), dense bodies (D), dilated rER cisternae, and mitochondria (M). Note the presence of some loss in the free ribosomes (r).(x4800) Inset: Shows synapses on the cells of the ventromedial nucleus in hypothalamus of treated newborn rat. Note the extensive loss of synaptic vesicles and the presence of damaged mitochondria (M) in the presynaptic terminal (arrow). Arrow head points to the synaptic contact with cell. (x19000).

Morphometric analysis

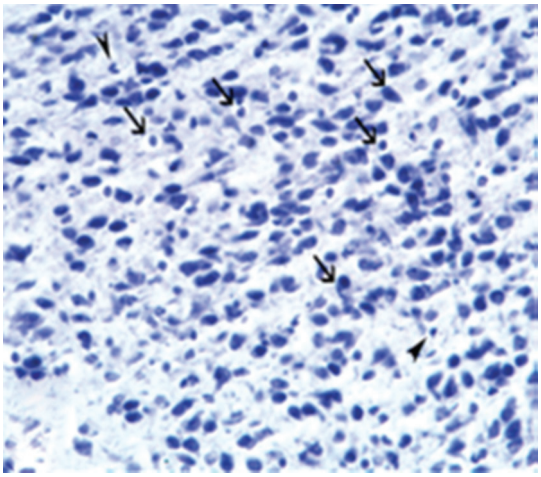
In newborn rats, the mean number of cells in the ventromedial nucleus per an area of 12 360 μm^2 in the offspring of diabetic mothers was found to be 170 ± 3.7, which shows an insignificant difference as compared with the offspring of control mothers where the mean number was 173 ± 2.3. At the ages of 21 days and 2 months, the mean numbers of cells in the ventromedial

Table 2 The mean number of cells in the ventromedial nucleus per an area of 12 360 μm² of the offspring of control mothers and that of diabetic mothers at different ages

Ages	Offspring of control mothers			Offspring of diabetic mothers			Difference	*t*
	n	Mean	SE	*n*	Mean	SE		
Newborn	5	173.4	2.315	5	170.4	3.696	3	0.688
21 days	5	200	3.536	5	163.4	2.561	36.6	8.383**
2 months	5	175.4	1.86	5	116	1.703	59.4	23.554**

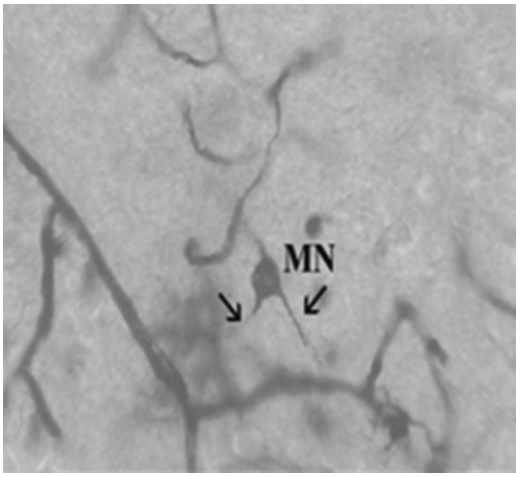
**$P<0.01$, highly statistical significance difference.

Figure 32



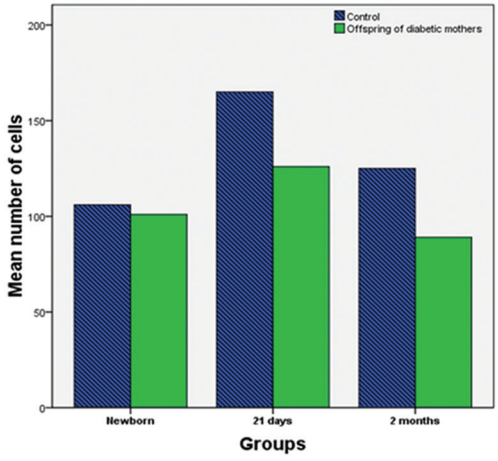
A photomicrograph showing the ventromedial nucleus in the hypothalamus of twenty-one days old offspring of diabetic mothers. Most of the cells have lysis of cytoplasm and darkly stained nuclei (arrows). Some cells have pyknotic nuclei (arrow heads). (Gallocyanin stain ×400).

Figure 33



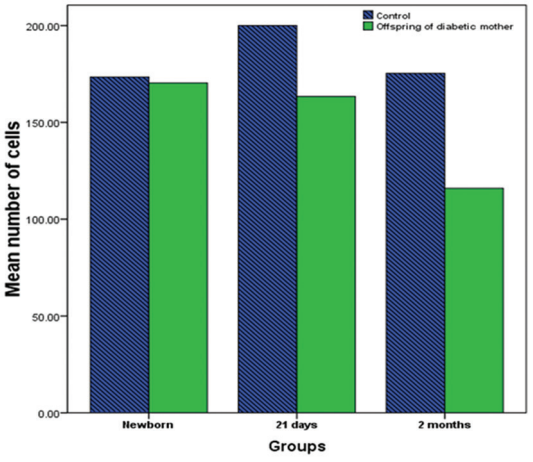
A photomicrograph showing the ventromedial nucleus in the hypothalamus of twenty one days old rat offspring of diabetic mother. Note the decrease in the extension of dendrites (arrows) of a multipolar neuron (MN). (Golgi-Cox stain ×250).

Histogram 1



Relationship between the number of cells of the paraventricular nucleus per an area of 12 360 μm2 in offspring of control mothers and offspring of diabetic mothers among different age groups.

Histogram 2



Relationship between the number of cells of the ventromedial nucleus per an area of 12 360 μm2 in offspring of control mothers and offspring of diabetic mothers among different age groups.

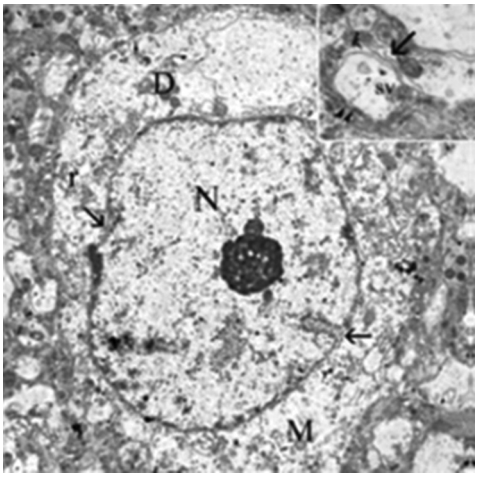
nucleus in the offspring of diabetic mothers were found to be 163 ± 2.6 and 116 ± 1.7, respectively, which show a highly significant decrease ($P < 0.01$) as compared with the corresponding offspring of control mothers where the mean numbers were 200 ± 3.5 and 175 ± 0.7, respectively (Table 2 and Histogram 2).

Discussion

Development of paraventricular and ventromedial hypothalamic nuclei

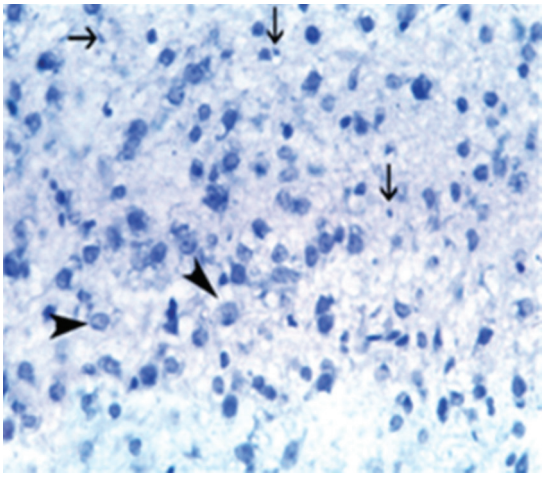
The present study showed that the paraventricular nucleus in the newborn rat appeared to be differentiated into two parts – dorsolateral (magnocellular part) and

Figure 34



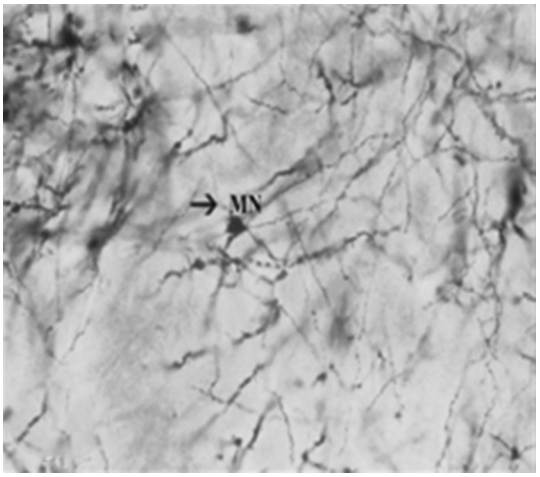
An electron photomicrograph of the ventromedial nucleus cell in hypothalamus of twenty-one days old rat offspring of diabetic mother. The nucleus (N) shows marked irregularities of the nuclear membrane(arrows). The cytoplasm appears to be rarified. Many dense bodies (D), some free ribosomes (r) and mitochondria (M) can be seen. (x4800) Inset: Shows synaptic contact (arrow head) on the cells of ventromedial nucleus in hypothalamus of treated twenty one days old rat. Loss of synaptic vesicles (sv) can be observed in the presynaptic terminal (arrow). (x19000).

Figure 35



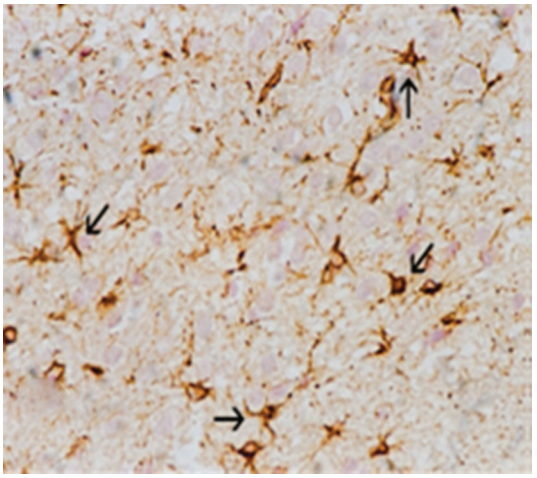
A photomicrograph showing the ventromedial nucleus in the hypothalamus of two months old offspring of diabetic mothers. Note the presence of some cells that show lysis of cytoplasm and pyknotic nuclei (arrows). Some cells appear faintly stained(arrowheads). (Gallocyanin stain ×400).

Figure 36



A photomicrograph showing the ventromedial nucleus in the hypothalamus of two months old rat offspring of diabetic mother. Note the decrease in the extension of dendrites (arrows)in multipolar neuron(MN). (Golgi-Cox stain ×250).

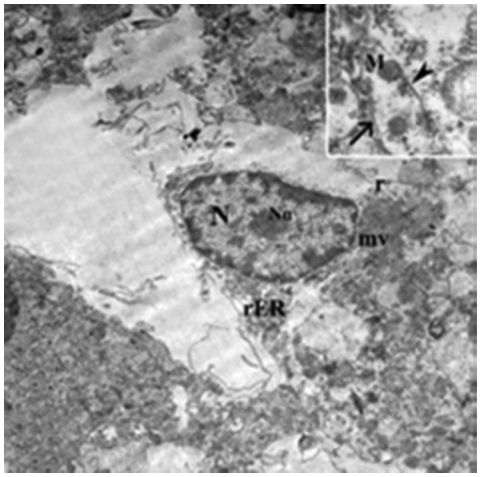
Figure 37



A photomicrograph showing the ventromedial nucleus in the hypothalamus of two months old rat offspring of diabetic mother. Note the presence of many astrocytes that have branched processes (arrows) (Anti GFAP immunostaining ×400).

ventromedial (parvocellular part). In the following age groups, the difference between the two divisions became apparent. The magnocellular part was formed of large cells with prominent nuclei, whereas the parvocellular part was formed of small packed cells. At the age of 2 months, the cells in the magnocellular part became packed with Nissl substance, whereas the neurons of the parvocellular part possessed scanty cytoplasm. Ultrastructurally, the present study showed that the neurons in the magnocellular part have rounded euchromatic nuclei, whereas the parvocellular part showed neurons with oval nuclei. The cytoplasm was rich in free ribosomes, rough endoplasmic reticulum, and mitochondria. These findings are similar to those of Simmons and Swanson [7] who found that the neurons in the paraventricular nucleus in rat fall into three major categories. The first group is the magnocellular neuroendocrine neurons that send their axons directly to the posterior lobe of the pituitary and secrete either vasopressin or oxytocin. The second group is the parvicellular neuroendocrine neurons that synthesize and secrete a variety of releasing or release-inhibiting hormones into the hypophysial portal system for transport to the anterior pituitary lobe to influence the synthesis and release of hormones from endocrine cells. The third group is the descending preautonomic neurons that send projections to the lower brainstem

Figure 38



An electron photomicrograph of the ventromedial nucleus cell in hypothalamus of two months old offspring of diabetic mother. Note that the nucleus shows peripheral chromatin condensation (N) and nucleolus (Nu). The cytoplasm appears to be rarified. Few ribosomes (r), dilated rough endoplasmic reticulum cisternae (rER) and multivesicular bodies can be observed (mv). (x4800) Inset: Shows synaptic contact (arrow head) on the cells of ventromedial nucleus in hypothalamus of treated two months old rat. Note the loss of synaptic vesicles and the destructed cristae of mitochondria(M) in the presynaptic terminal (arrow). (x19000).

and/or spinal cord and are presumably involved in coordinating both sympathetic and parasympathetic, as well as somatomotor, responses with endocrine activity. The paraventricular nucleus was described by Biag *et al.* [8] as one of the most complex, heterogeneously organized nuclei of the brain. In addition, McClellan *et al.* [9] described that the development of the hypothalamic paraventricular nucleus was found to involve several factors that work together to establish a cell group that regulates neuroendocrine functions and behaviors.

The ventromedial nucleus in this study was observed to be well defined at birth. It was oval in shape, consisting of rounded cells of variable sizes. At the age of 21 days, it became separated from the surrounding tissue by a cell–sparse halo. These observations were supported by Crandall *et al.* [10] who described that the ventromedial nucleus was strongly defined by the surrounding cell-poor, fiber-rich zone, and this zone was rich in dendritic processes. These results were also supported by McClellan *et al.* [11] who defined the ventromedial nucleus as a medial hypothalamic cell group that sits close to the base of the diencephalon, adjacent to the third ventricle above the median eminence and pituitary complex. They added that it was a bilateral cell group that had an elliptical shape, stretching more laterally in rodents as it extends rostral to caudal. At the age of 21 days, the cells reached adult appearance cytologically as described by Pozzo Miller and Aoki [12].

Choi and Dallman [13] found that the role of the ventromedial nucleus in the control of multiple processes and behaviors is complex because of its close interconnection with other sites, including the amygdala and the preoptic area. However, more recent studies [14,15] including genetic mouse models suggested roles for the ventromedial nucleus in the regulation of energy balance.

In the present study, examination of the sites of contacts (synapses) between the nerve processes and the neurons in both the paraventricular and the ventromedial nuclei in different age groups revealed the presence of synaptic density, well-distributed synaptic vesicles, and mitochondria. These were in accordance with the study by Harris and Weinberg [16] who described in detail the structure of synapse in mammalian brain. They reported that the active zone is a specialized region on the presynaptic plasma membrane, where synaptic vesicles are docked and primed for release. The active zone was opposite to the postsynaptic density. They added that dendrites, axons, and astroglial processes form a fine felt-like mesh (the neuropil) where most of the synaptic interactions occur. White and Barone [17] stated that normal development of the vertebrate nervous system requires the formation of synapses, which are regulated by neurotropins. A second process removes unnecessary neurons by apoptosis, leaving a more efficient synaptic configuration. Several agents are able to disturb the developing process, resulting in altered cell number and neural function.

The effect of maternal-induced diabetes on the development of the paraventricular and ventromedial hypothalamic nuclei

Witkop *et al.* [18] stated that when a fetus (over 12th week of gestation) is exposed to high levels of maternal glucose, it responds by secreting high levels of insulin in its circulation to control hyperglycemia. This is a 'double sword' mechanism where insulin that also has growth hormone properties develops a high tendency for fetal macrosomia and an increased rate of delivery complications. In accordance with Kitzmiller *et al.* [19], glucose passes from maternal to fetal circulation through the placental barrier, while maternal insulin does not. Therefore, maternal insulin concentrations do not have a direct effect on fetal glucose and insulin metabolism, but only an indirect one by influencing maternal glucose levels [20]. Consequently, Franke *et al.* [21] found that induced maternal hypoinsulinemia as well as its normalization by islet transplantation were unlikely to have direct effects on the fetus.

This study revealed that the hypothalamus of pups of diabetic mothers in the newborn period had paraventricular and ventromedial nuclei that showed cells with darkly stained nuclei. At the age of 21 days, most cells showed the presence of pyknotic nuclei, and at the age of 2 months nearly all the cells had pyknotic nuclei. In the ventromedial nucleus, many cells showed lysis of cytoplasm. Ultrastructural examination of the paraventricular and ventromedial nuclei revealed the presence of chromatin condensation in neuronal nuclei. The presence of many damaged mitochondria and marked reduction in the amount of ribosomes were also observed. These findings are in agreement with Plagemann *et al.* [22], who revealed that in 21-day-old offspring of diabetic rats the paraventricular and ventromedial nuclei showed clear reductions in the mean area of neuronal nuclei and area of neuronal cytoplasm. They also identified structural disorganization of the ventromedial nucleus as an early phenomenon occurring after intrauterine and neonatal hyperglycemia and consecutive hyperinsulinism. In addition, Khaksar *et al.* [23] found an impairment of the mitochondrial cristae and the mitochondria showed signs of vacuolation on examination of neurons in offspring of diabetic mothers.

Ultrastructural examination of the site of synapse between nerve processes and neurons in this study showed a decrease in the amount of synaptic vesicles and destructed mitochondria in the presynaptic terminal making synaptic contact with cells of the paraventricular and ventromedial nuclei. These changes were observed in the newborn, at 21 days, and 2 months of age. These results are in accordance with Hernandez-Fonseca *et al.* [24], who reported that destructed mitochondria suggest an altered synaptic transmission and could contribute to abnormal synaptic plasticity and cognitive impairments observed in experimental diabetes. Consistent with these findings, Magarinos and Mac Ewen [25] found that there was dispersion of synaptic vesicles in the presynaptic hippocampal mossy fiber terminals in 9-day-old diabetic rats.

The Golgi–Cox technique showed a decrease in the extension and in the branching of the dendrites in cells of the studied hypothalamic nuclei in offspring of diabetic mothers in comparison with the control groups. These observations were supported by Jing *et al.* [26] who suggested that maternal hyperglycemia can retard dendritic development in the fetal brain, and that these changes partially resulted from abnormal insulin/insulin-like growth factor-I signaling in the fetal brain.

The morphometric study showed that both the paraventricular and the ventromedial nuclei showed a highly significant decrease in the number of cells at the ages of 21 days and 2 months in the offspring of diabetic mothers in comparison with the offspring of diabetic mothers. In support of these findings, Dörner *et al.* [27] observed a decreased numerical density and a decreased total number of neurons in the ventromedial nucleus in offspring of diabetic mothers, which persisted from weaning (day 21 of life) into adulthood.

Expression of GFAP, an intermediate filament protein, is commonly used as a marker of astrocytes [28]. For many years, extensive studies of astrocyte–neuron interactions have been carried out in a number of brain areas as noted by Gerics *et al.* [29]. In control rats, this study revealed a scanty number of astrocytes seen in the ventromedial and paraventricular nuclei. These results were in accordance with Zilles *et al.* [30] who found that in the hypothalamus of adult rats, the number of GFAP-expressing astrocytes was highest in the periventricular region, falling with increasing distance from the third ventricle, with nearly no GFAP immunoreactivity in the ventromedial nucleus, dorsomedial nucleus, and lateral hypothalamic area.

This study demonstrated an increase in the expression of GFAP in the paraventricular and ventromedial nuclei in the adult offspring of diabetic rats. These findings can be explained by the reactive astrogliosis that occurs in response to inflammation and injury to the central nervous system as reported by Buckman *et al.* [28] who defined astrogliosis as hypertrophy and hyperplasia of astrocytes in response to acute or chronic insults to the brain. Chronic fetal hypoxia present in maternal diabetes mellitus may increase the inflammatory burden incurred by the fetus [31] and the produced inflammatory cytokines affect neuronal development [32]. In support of the present results, Magarinos and Mac Ewen [25] reported that dysfunctional glucose regulation and/or insufficient insulin availability elicit neuronal synaptic reorganization in experimental diabetic rodents [33]. Theodosis and MacVicar [34] reported that astrocytes interact with hypothalamic neurons to modulate neuronal maturation and neuroendocrine activity. In addition, astrocytes are known to regulate synaptic plasticity in the hypothalamo–neurohypophysial system [35]. Therefore, alterations in the number of astrocytes as well as a disturbed glia-to-neuron ratio and glia–neuron interaction might have permanent consequences for hypothalamic organization and neuroendocrine functions. These changes could be explained by mechanisms such as hypoxia and oxidative stress, especially through the hexosamine biosynthesis pathway, which might be involved in diabetes embryopathy, by inducing apoptosis during critical phases of organ development [36].

The critical period for hypothalamic development in the rat is in the first 21 days of life as documented by Pozzo Miller and Aoki [12]. In addition, disturbance in catecholamines of the hypothalamic nuclei has a role in explaining the effect of maternal diabetes on the development of the hypothalamic nuclei [37]. Plagemann *et al.* [22] reported that impaired function of the ventromedial nucleus may contribute to the development of hyperphagia, obesity, and diabetogenic disturbances in the offspring of gestational diabetic rat dams. Aside from the paraventricular nucleus' role in body weight control, Dampney *et al.* [38] reported that the paraventricular nucleus was crucially involved in the regulation of blood pressure. Therefore, malorganization of this nucleus might also contribute to long-term cardiovascular disturbances.

Leloup [39] reported that insulin binding was increased in hypothalamic-related and extrahypothalamic-related nuclei in the offspring of hyperglycemic mothers. This increase was specific to areas involved in the nervous control of metabolism and could be a factor in glucose intolerance and impairment of insulin secretion exhibited by young, adult rats from hyperglycemic mothers. Plagemann [3] added that elevated insulin levels in fetal and perinatal life (hyperinslinemia) are pathognomonic in children of mothers with diabetes during pregnancy (type 1 diabetes, type 2 diabetes, gestational diabetes).

Conclusion

This study demonstrated that maternal diabetes can structurally affect the development of the ventromedial and paraventricular hypothalamic nuclei. These changes can suggest the possibility of insulin resistance and diabetes in the offspring of diabetic mothers. It is recommended that there should be strict control of diabetes before pregnancy. Women with diabetes mellitus who are of reproductive age should be identified as members of a high-risk group, and intensive glycemic control before conception and throughout pregnancy is a necessity.

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Grants office Faculty of Medicine Assiut University.

Conflicts of interest

There are no conflicts of interest.

References

1 Caqueret A, Yang C, Duplan S, Boucher F, Michaud JL. Looking for trouble: a search for developmental defects of the hypothalamus. Horm Res 2005; 64:222–230.

2 Vambergue A, Fajardy I. Consequences of gestational and pregestational diabetes on placental function and birth weight. World J Diabetes 2011; 2:196–203.

3 Plagemann A. Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. Physiol Behav 2006; 86:661–668.

4 Wang J-Q, Yin J, Song Y-F, Zhang L, Ren Y-X, Wang D-G, Jing Y. Brain aging and AD-like pathology in streptozotocin-induced diabetic rats. J Diabetes Res 2014; 2014:796840.

5 Hami J, Shojae F, Vafaee-Nezhad S, Lotfi N, Kheradmand H, Haghir H. Some of the experimental and clinical aspects of the effects of the maternal diabetes on developing hippocampus. World Jo Diabetes 2015; 6:412–422.

6 Drury RAB, Wallington EA. Preparation and fixation of tissues. In: Drury RAB, Wallington EA, editors. *Carleton's histological technique*. Vol. 5. Oxford: Oxford University Press; 1980. pp. 41–54.

7 Simmons DM, Swanson LW. Comparison of the spatial distribution of seven types of neuroendocrine neurons in the rat paraventricular nucleus: toward a global 3D model. J Comp Neurol 2009;516:423–441.

8 Biag J, Huang Y, Gou L, Hintiryan H, Askarinam A, Hahn JD, Toga AW, Dong H-W. Cyto- and chemoarchitecture of the hypothalamic paraventricular nucleus in the C57BL/6J male mouse: a study of immunostaining and multiple fluorescent tract tracing. J Comp Neurol 2012; 520: 6–33.

9 McClellan KM, Stratton MS, Tobet SA. Roles for gamma-aminobutyric acid in the development of the paraventricular nucleus of the hypothalamus. J Comp Neurol 2010; 518:2710–2728.

10 Crandall JE, Tobet SA, Fischer I, Fox TO. Age-dependent expression of microtubule-associated protein 2 in the ventromedial nucleus of the hypothalamus. Brain Res Bull 1989; 22:571–574.

11 McClellan KM, Parker KL, Tobet S. Development of the ventromedial nucleus of the hypothalamus. Front Neuroendocrinol 2006; 27:193–209.

12 Pozzo Miller LD, Aoki A. Postnatal development of the hypothalamic ventromedial nucleus: neurons and synapses. Cell Mol Neurobiol 1992; 12:121–129.

13 Choi S, Dallman MF. Hypothalamic obesity: multiple routes mediated by loss of function in medial cell groups. Endocrinology 1999; 140:4081–4088.

14 King BM. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. Physiol Behav 2006; 87:221–244.

15 Majdic G, Young M, Gomez-Sanchez E, Anderson P, Szczepaniak LS, Dobbins RL, *et al.* Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 2002; 143:607–614.

16 Harris KM, Weinberg RJ. Ultrastructure of synapses in the mammalian brain. Cold Spring Harb Perspect Biol 2012; 4:a005587.

17 White LD, Barone SJ. Qualitative and quantitative estimates of apoptosis from birth to senescence in the rat brain. Cell Death Differ 2001; 8:345–356.

18 Witkop CT, Neale D, Wilson LM, Bass EB, Nicholson WK. Active compared with expectant delivery management in women with gestational diabetes: a systematic review. Obstet Gynecol 2009; 13:206–217.

19 Kitzmiller JL, Buchanan TA, Kjos S, Combs CA, Ratner RE. Pre-conception care of diabetes, congenital malformations, and spontaneous abortions. Diabetes Care 1996; 19:514–541.

20 Weiss PAM. Gestational diabetes: a survey and the Graz approach to diagnosis and therapy. In: *Gestational diabetes*. Weiss PAM, Coustan DR, editors. Wien: Springer; 1988. pp. 1–58.

21 Franke K, Hardera T, Aertsb L, Melchiora K, Fahrenkroga S, Rodekampa E, *et al.* Programming of orexigenic and anorexigenic hypothalamic neurons in offspring of treated and untreated diabetic mother rats. Brain Res 2005; 1031:276–283.

22 Plagemann A, Harder T, Janert U, Rake A, Rittel F, Rohde W, Dorner G. Malformations of hypothalamic nuclei in hyperinsulinaemic offspring of gestational diabetic mother rats. Dev Neurosci 1999; 21:58–67.

23 Khaksar Z, Jelodar G, Hematian H. Ultra-structural changes in cells from the CNS in offspring from diabetic rats. Comp Clin Path 2012; 21:1203–1206.

24 Hernandez-Fonseca JP, Rincon J, Pedreañez A, Viera N, Arcaya JL, Carrizo E, Mosquera J. Structural and ultrastructural analysis of cerebral cortex, cerebellum, and hypothalamus from diabetic rats. Exp Diabetes Res 2009; 2009:329632.

25 Magarinos AM, Mac Ewen BS. Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress, Proc Natl Acad Sci USA 2000; 97:11056–11061.

26 Jing YH, Song YF, Yao YM, Yin J, Wang DG, Gao LP. Retardation of fetal dendritic development induced by gestational hyperglycemia is associated with brain insulin/IGF-I signals. Int J Dev Neurosci 2014; 37:15–20.

27 Dörner G, Plagemann A, Rückert J, Götz F, Rohde W, Stahl F, *et al.* Teratogenetic maternofetal transmission and prevention of diabetes susceptibility. Exp Clin Endocrinol 1988; 91:247–258.

28 Buckman LB, Thompson MM, Moreno HN, Ellacott KLJ. Regional astrogliosis in the mouse hypothalamus in response to obesity. J Comp Neurol 2013; 521:1322–1333.

29 Gerics B, Szalay F, Hajós F. Glial fibrillary acidic protein immunoreactivity in the rat suprachiasmatic nucleus: circadian changes and their seasonal dependence. J Anat 2006; 209:231–237.

30 Zilles K, Hajos F, Kalman M, Schleicher A. Mapping of glial fibrillary acidic protein-immunoreactivity in the rat forebrain and mesencephalon by computerized image analysis. J Comp Neurol 1991; 308:340–355.

31 Loukovaara M, Leinonen P, Teramo K, Alfthan H, Stenman UH, Andersson S. Fetal hypoxia is associated with elevated cord serum C-reactive protein levels in diabetic pregnancies. Biol Neonate 2004; 85:237–242.

32 Zalcman S, Green-Johnson JM, Murray L, Nance DM, Dyck D, Anisman H, Greenberg AH. Cytokine-specific central monoamine alterations induces by IL-1, -2 and -6. Brain Res 1994; 643:40–49.

33 Saravia FE, Revsin Y, Gonzalez Deniselle MC, Gonzalez SL, Roig P, Lima A. Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice. Brain Res 2002; 957:345–353.

34 Theodosis DT, MacVicar B. Neurone-glia interactions in the hypothalamus and pituitary. Trends Neurosci 1996; 19:363–367.

35 Panatier A. Glial cells: indispensable partners of hypothalamic magnocellular neurones. J Neuroendocrinol 2009; 21:665–672.

36 Simeoni U, Barker DJ. Offspring of diabetic pregnancy: long-term outcomes. Semin Fetal Neonatal Med 2009; 14:119–124.

37 Plagemann A, Harder T, Lindner R, Melchior K, Rake A, Rittel F, *et al.* Alterations of hypothalamic catecholamines in the newborn offspring of gestational diabetic mother rats A. Brain Res Dev Brain Res 1998; 109: 201–209.

38 Dampney RAL, Coleman MJ, Fontes MAP, Hirooka Y, Horiuchi J, Li YW, *et al.* Central mechanisms underlying short- and long-term regulation of the cardiovascular system. Clin Exp Pharmacol Physiol 2002; 29:261–268.

39 Leloup C, Magnan C, Alquier T, Mistry S, Offer G, Arnaud E, *et al.* Intrauterine hyperglycemia increases insulin binding sites but not glucose transporter expression in discrete brain areas in term rat foetuses. Pediatr Res 2004; 56:263–267.